

Fig. S1.

Dark CPD production in melanocytes.

(A) In NIH3T3 murine fibroblasts, CPD are induced by UVA at time 0 and are then repaired. CPD were assayed by ELISA.

(B) Dark CPD are detected in UVA-exposed C57BL/6 melanocytes when assayed using UVDE endonuclease to nick DNA at CPD and alkaline comet to detect nicks.

(C) Dark CPD in cell nuclei are detected after UVA exposure of melanin-containing C57BL/6 melanocytes but not in melan-c albino melanocytes. CPD were visualized by immunofluorescence using anti-CPD antibody.

- (D) Quantitation of anti-CPD immunofluorescence in cells of panel (C).
- (E, F) Narrowband UVB exposure does not cause dark CPD in murine fibroblasts or albino melanocytes. CPD were assayed by ELISA.
- (G) Dark CPD in UVB-exposed C57BL/6 melanocytes. CPD were assayed by ELISA.
- (H) Comparable levels of dark CPD are seen after UVA and UVB in cultures of melanin-containing C57BL/6 melanocytes at doses giving similar survival (~75% and ~85%, respectively).
- (I) Dark CPD production after UVB, for six Caucasian human donors. CPD were assayed by ELISA and the data were normalized to the 0 hr value for each donor.
- p values by t-test are for the difference between the asterisked timepoint and 0 h or as indicated. *, p ≤ 0.05; ** 0.005; *** 0.0005; **** 0.00005.

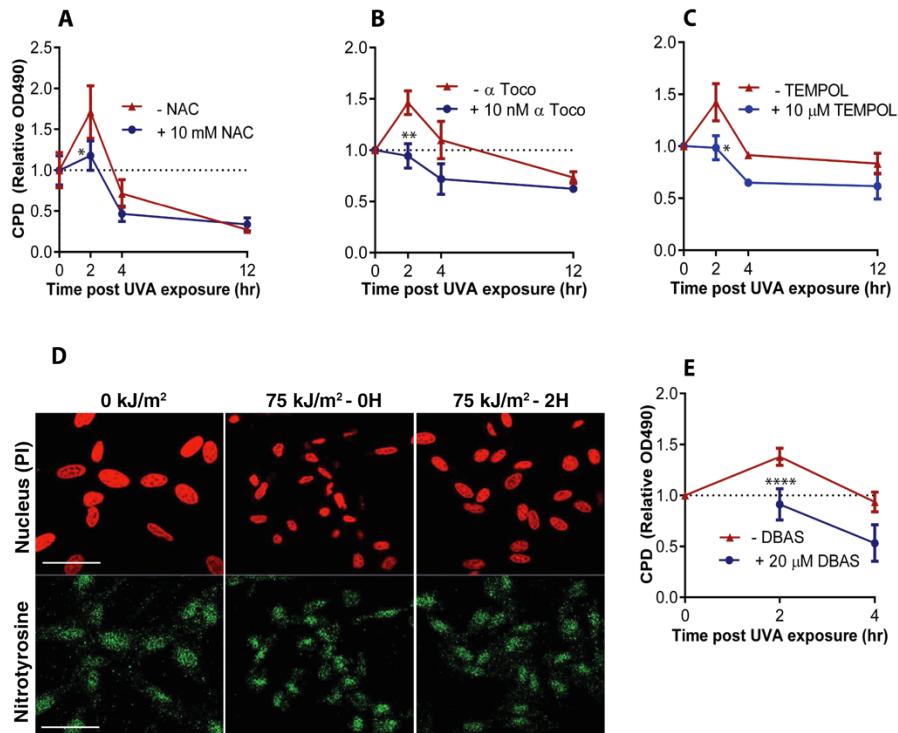


Fig. S2

Dark CPD are blocked by scavengers of reactive oxygen species, they correlate with elevated peroxynitrite, and they require triplet states.

- (A) N-acetyl cysteine (NAC).
- (B) α -tocopherol (vitamin E).
- (C) TEMPOL (4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl), a superoxide scavenger.
- (D) Basal and UVA-induced peroxynitrite activity in the nuclei of human melanocytes, assessed as 3-nitrotyrosine in proteins.
- (E) Dark CPD are blocked by the triplet energy acceptor DBAS (9,10-dibromoanthracene-2-sulfonate anion).

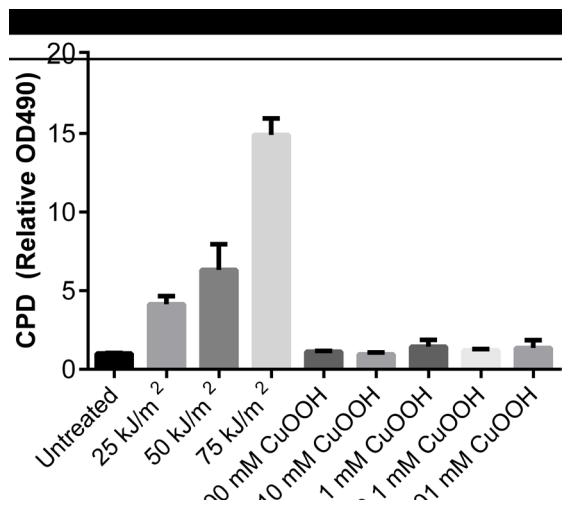


Fig. S3

Lipid peroxidation does not appear to be the source of electronically-excited triplet states that create dark CPD in DNA.

Nuclei from C57BL/6 melanocytes were incubated with cumene hydroperoxide (CuOOH) as a lipid peroxidant and nuclear DNA was assayed for CPD using ELISA. Data are from two experiments.

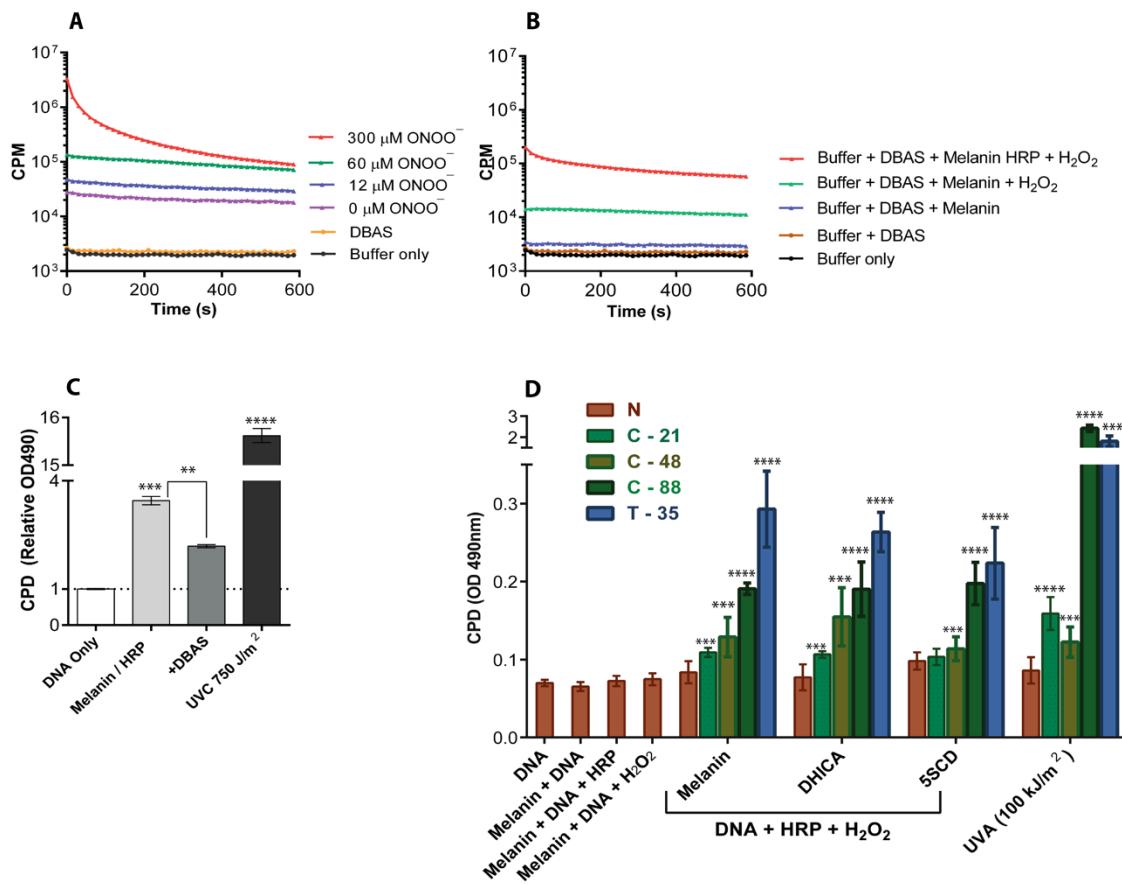


Fig. S4

Reconstitution of chemiexcitation in a cell-free system.

(A, B) Ultraweak chemiluminescence is generated in the presence of DBAS as a triplet energy acceptor, when synthetic melanin is oxidized with:

- (A) peroxynitrite or
- (B) horseradish peroxidase plus H₂O₂, indicating the involvement of electronically-excited triplet states.

(C) Cyclobutane pyrimidine dimers (CPD) are created in pUC19 DNA in the dark when melanin is oxidized by horseradish peroxidase plus H₂O₂. Diverting triplet energy to luminescence with DBAS blocks the production of CPD, confirming that excited triplet states in melanin create CPD by energy transfer in the dark. Also shown is the level of CPD induced by direct DNA absorption of germicidal UVC; this dose is ~100 fold higher than that lethal to 50% of normal cells, so even lower CPD levels are biologically relevant.

(D) Cytosine-containing CPD are created in the dark during the *in vitro* reaction of oligonucleotides with horseradish peroxidase plus H₂O₂. Oligonucleotides were constructed to contain: N oligo, no dipyrimidine sites; C-21, 21 TC plus CT sites; C-48, 48 TC, CT, and CC sites; C-88, 88 TC, CT, and CC sites containing the melanoma mutation hotspot motif; T-35, 35 TT sites (see Methods). Oligonucleotides were treated with horseradish peroxidase plus H₂O₂ in the presence of synthetic

melanin, DHICA, or 5SCD without UV or were irradiated with 100 kJ/m² UVA as a positive control. CPD were measured by ELISA; data are from 3 repeat experiments.

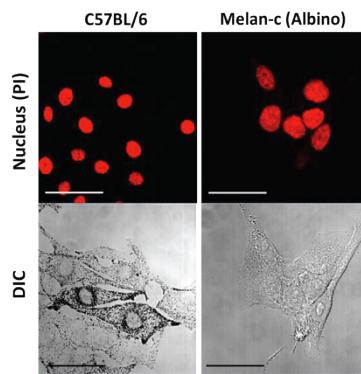


Fig. S5

Differential interference contrast (DIC) microscopy imaging of melanin granules in melanocytes.

DIC visualizes granules in the cytoplasm and perinuclear region of melanin-containing melanocytes but not in albino melanocytes. These are presumed to be melanin aggregates in melanosomes, coated vesicles, and endoplasmic reticulum because they have the same size and cellular locations as granules seen in melanocytes that have been immunostained for tyrosinase. After UVA exposure, many granules appear in the nucleus (Supplementary Movies S1 and S2).

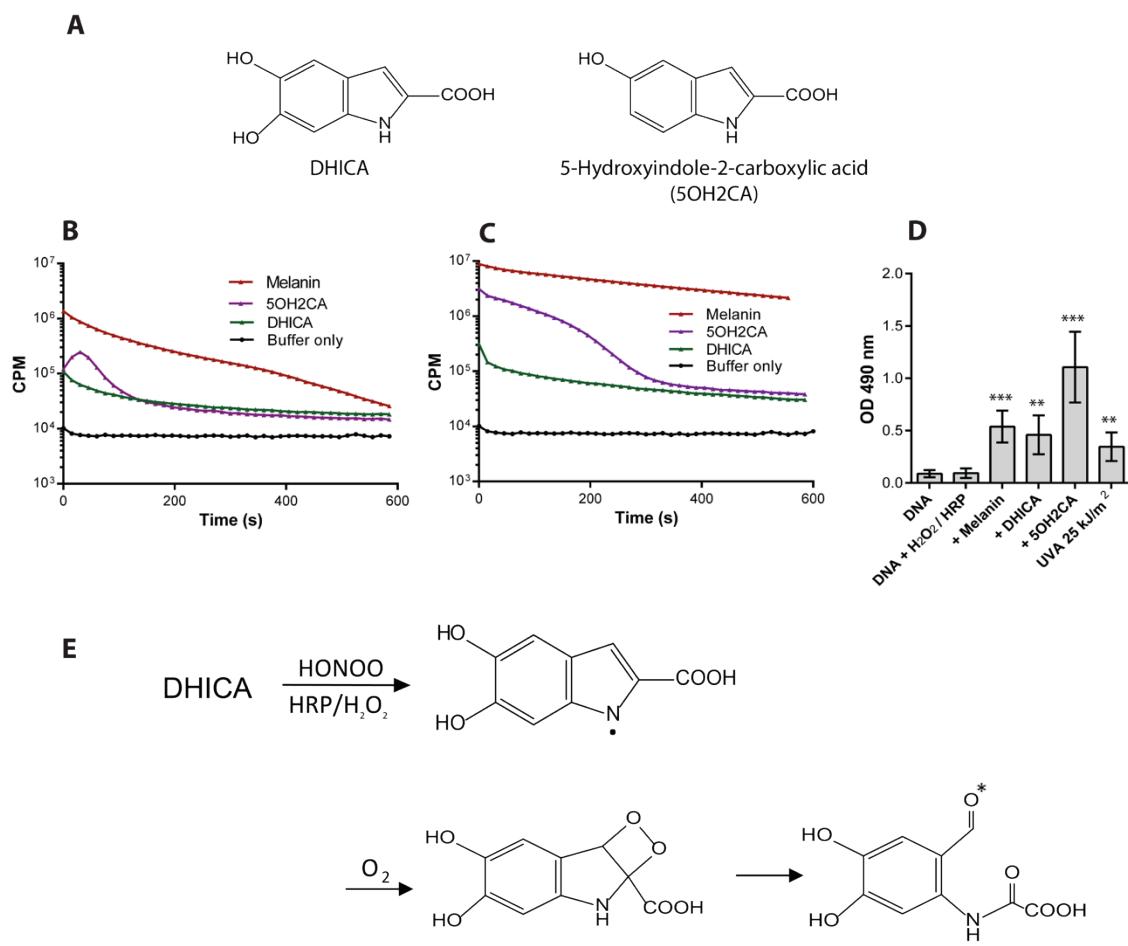


Fig. S6

The DHICA reaction does not require the 6-membered ring for chemiluminescence or generation of dark CPD.

- (A) The DHICA analog 5-hydroxyindole-2-carboxylic acid (5OH2CA, top right) lacks one OH, preventing a dioxetane from forming on the 6-membered ring by oxidation of the *o*-dihydroquinone and preventing structure 1 of Fig. 4.
- (B) Reacting 5OH2CA with horseradish peroxidase plus H₂O₂ results in chemiluminescence.
- (C) Reacting 5OH2CA with peroxy nitrite results in chemiluminescence.
- (D) Reacting 5OH2CA with horseradish peroxidase plus H₂O₂ generates CPD. These results suggest that structure 2 of Fig. 4 is the predominant product.
- (E) This reaction product would result from H abstraction at the indole N to create N[•], followed by resonance to C[•] at C3, addition of O₂, and subsequent cyclization (24). Dioxetanes then undergo spontaneous thermolysis to create a triplet-state carbonyl (structure 2).

Movie S1

Abundant melanin granules (white) surrounding the nucleus of a melanin-containing murine melanocyte not exposed to UVA. 3D reconstruction from stack of optically sectioned DIC micrographs of unfixed C57BL/6 melanocytes (see Methods).

Movie S2

Melanocyte 1 hr after exposure to 200 kJ/m^2 UVA, showing melanin granules dispersed within the nuclear volume.

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